Mechanisms for cholesterol homeostasis in rat jejunal mucosa: effects of cholesterol, sitosterol, and lovastatin

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Abstract The effects of feeding cholesterol, sitosterol, and lovastatin on cholesterol absorption, biosynthesis, esterification, and LDL receptor function were examined in the rat jejunal mucosa. Cholesterol absorption was measured by the dual-isotope plasma ratio method; the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, was measured as total and expressed enzyme activities (in the absence and presence of a phosphatase inhibitor, NaF, respectively); mucosal total and esterified cholesterol concentrations were determined by gas-liquid chromatography; LDL receptor function was assayed as receptor-mediated binding of ^{125}I-labeled LDL to mucosal membranes. Feeding 2% sitosterol or 0.04% lovastatin for 1 week significantly ($P < 0.01$) decreased the amounts of cholesterol absorbed per day (−85% and −63%, respectively). In contrast, feeding 2% cholesterol for 1 week increased the amounts of absorbed cholesterol 27-fold, even though the percent absorption significantly decreased. With all three treatments, there was a coordinate regulation of total HMG-CoA reductase activity and receptor-mediated LDL binding. Cholesterol feeding downregulated both total jejunal HMG-CoA reductase activity ($P < 0.05$) and receptor-mediated LDL binding ($P < 0.01$), whereas lovastatin- and sitosterol-supplemented diets significantly upregulated both of these parameters. In the control, cholesterol-fed, and sitosterol-fed animals, about half of the total jejunal HMG-CoA reductase activity was expressed (in functional dephosphorylated form). However, in the lovastatin-treated rats with 4-fold stimulation of HMG-CoA reductase, only 23% of the total enzyme activity was expressed. Changes in total HMG-CoA reductase activity and receptor-mediated LDL binding in all tested groups occurred with no change in total concentrations of mucosal cholesterol, and only cholesterol-fed animals had increased mucosal esterified cholesterol concentrations.⬛ Thus, in response to various fluxes of dietary or newly formed cholesterol, HMG-CoA reductase and receptor-mediated LDL binding are coordinately regulated to maintain constant cellular cholesterol concentrations in the jejunum.—Nguyen, L. B., S. Shefer, G. Salen, G. S. Tint, F. Ruiz, and J. Bullock. **Mechanisms for cholesterol homeostasis in rat jejunal mucosa: effects of cholesterol, sitosterol, and lovastatin.** *J. Lipid Res.* 2001. **42:** 195–200.

The intestine plays an important role in cholesterol homeostasis. It regulates the amount of dietary and endogenous biliary cholesterol that enters the body and has a high rate of cholesterol biosynthesis that, in some species and under certain physiological conditions, may exceed that of the liver (1, 2). In addition, intestinal mucosa is involved in cholesterol esterification (3) and synthesis of various apolipoproteins [apolipoprotein (apo) B-48, apoA-I, apoA-II, and apoA-IV] (4–7). It is also a site for intracellular assembly and release of chylomicrons, nascent high density lipoprotein (HDL), and an intestinal form of very low density lipoprotein (8, 9).

De novo cholesterol synthesis is known to contribute two to three times more cholesterol to the total body pool than does the absorption of dietary cholesterol, which occurs predominantly in the jejunum (10). In the intestinal mucosa, as in other tissues, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) is the rate-limiting step in cholesterol synthesis and its activity correlates with the cholesterol synthetic rate (11, 12). This microsomal enzyme has a diurnal rhythm that coincides with the diurnal variation of the hepatic HMG-CoA reductase (13), is stimulated by biliary diversion or sitosterol feeding, and is inhibited in rats fed cholesterol combined with bile acids (14).

The role of intestinal lipoprotein receptors in the regulation of cholesterol metabolism has been implicated in studies in which intestinal cholesterol synthesis was decreased in parabiont animals fed a control diet but ex-

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posed to high levels of plasma cholesterol (15). Suzuki et al. (16) and Sviridov et al. (17) reported specific and saturable binding, internalization, and degradation of low density lipoprotein (LDL) and HDL by rat and human intestinal cells. Spady, Bilheimer, and Dietschy (18), who measured the rates of uptake of labeled LDL by various tissues in the hamster, reported that the jejunum and ileum combined accounted for 7% of whole animal LDL clearance. Fong et al. (19) demonstrated by immunohistochemistry that LDL receptors were present throughout the rat intestine. However, there is still uncertainty whether LDL receptor function in the intestinal mucosa is regulated when changing needs for cellular cholesterol are met by changes in the rates of cholesterol biosynthesis (20).

The objectives of this study were to *1*) examine the effects of three known modulators of cholesterol metabolism (cholesterol, sitosterol, and lovastatin) on cholesterol absorption and mucosal cholesterol concentrations, and *2*) evaluate mechanisms for cholesterol homeostasis in the rat jejunum (cholesterol biosynthesis, esterification, and receptor-mediated LDL uptake) in response to changes in absorbed or endogenously formed cholesterol.

MATERIALS AND METHODS

Animals and tissue sample preparations

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200–250 g were fed ad libitum for 7 days a ground rodent chow diet (containing 0.027% cholesterol; Purina Mills, St. Louis, MO) without or with supplementation with 2% cholesterol (>99% pure; Sigma, St. Louis, MO), 2% sitosterol (gift from Eli Lilly, Indianapolis, IN), or 0.04% lovastatin (gift from Merck Pharmaceuticals, New Brunswick, NJ). The rats were weighed at the start and end of the feeding period. All treatment groups were matched for body weight and feeding periods, consumed similar amounts of food, and gained similar weight.

The jejunal segments were obtained by discarding the first 10 cm from the stomach and using the proximal third of the remaining intestinal tract. The jejunal segments were flushed with cold saline containing 10 mM dithiothreitol. The mucosal cells were obtained by the scraping technique (21); homogenates of whole mucosal cells and microsomal fractions were prepared by sequential centrifugation in the presence and absence of a phosphatase inhibitor (sodium fluoride), as previously described (22). Small aliquots (200 µl) of the homogenates were saved for the determination of free and esterified cholesterol concentrations. Microsomes from each rat (9,000 *g*–100,000 *g* mucosal fraction) were used to prepare membranes for the measurements of receptor-mediated LDL binding. This cellular fraction was washed in buffer A {20 mM Tris [tris(hydroxymethyl)aminomethane], 50 mM NaCl, 1 mM CaCl$_2$, pH 7.5}, centrifuged at 100,000 *g* for 60 min at 4°C, and resuspended in buffer B (50 mM Tris, 100 mM NaCl, 0.5 mM CaCl$_2$, pH 7.5) at a protein concentration (23) of 2–6 mg/ml.

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey (Newark, NJ).

Cholesterol absorption

Cholesterol absorption, expressed as a percentage of dietary cholesterol, was measured by the dual-isotope plasma ratio method of Zilvermit and Hughes (24). [4-14C]cholesterol (specific activity, 53.1 mCi/mmol) and [1,2-3H]cholesterol (specific activity, 58 Ci/mmol) were obtained from DuPont New England Nuclear (Boston, MA). After 4 days on a test diet, the rats were administered [4-14C]cholesterol (6 µCi mixed with 20 mg of unlabeled cholesterol and sonicated in skim milk in a total volume of 0.5 ml per rat) by stomach tubing. [1,2-3H]cholesterol (3 µCi in 30 µl of ethanol, suspended in saline; total volume, 0.5 ml per rat) was immediately injected via the femoral vein under light anesthesia. The rats were maintained on the same test diet for an additional 3 days before cardiac puncture to collect 0.5–1 ml of blood. The ratio of 14C and 3H radioactivity in plasma was determined and compared with the ratio measured in the administered solutions. In preliminary experiments, where radioactivity in the plasma was determined 24, 48, and 72 h after oral and intravenous administration of the labeled cholesterol, constant percent cholesterol absorption was observed. This indicated that equilibrium had been reached after 24 h.

Total and esterified cholesterol concentrations in jejunal mucosal cells

Total cholesterol was extracted from homogenates of jejunal mucosal cells with 20 volumes of Folch solvent (chloroform–methanol 2:1, v/v) after addition of [1,2-3H(N)]cholesterol for internal recovery standard. Free and esterified cholesterol were separated by thin-layer chromatography (25), and eluted from the silica gel with ethyl acetate–methanol 85:15 (v/v). The esterified cholesterol fractions were dried under nitrogen, saponified in 5% KOH at 70°C for 1 h, and extracted with hexane. Both free and esterified cholesterol extracts were analyzed by capillary gas-liquid chromatography (25).

Receptor-mediated LDL binding to mucosal membranes

LDL (1.019 < d < 1.063) was isolated from venous blood of normal human volunteers by differential ultracentrifugation (26) and labeled with 125I by the monohydrochloride method (27). Binding of LDL to rat jejunal mucosal membranes was assayed by methods previously described (28). Receptor-mediated LDL binding to the mucosal membranes was determined as the difference between total binding of 125I-labeled LDL (assayed in the absence of unlabeled LDL) and nonspecific binding (assayed in the presence of a 40-fold excess unlabeled LDL). Previous work has shown that rat LDL receptors could specifically bind human LDL (16). Receptor-mediated LDL binding to mucosal membranes from the rat jejunum represented about 50% of total binding, was linear for at least 60 min and up to a protein concentration of 2 mg/ml, and showed a half-V_{max} at a concentration of 125I-labeled LDL of 25 µg/ml (**Fig. 1**).

Microsomal HMG-CoA reductase activity

The assay for total and expressed microsomal HMG-CoA reductase activity in the rat jejunal mucosa was carried out in the absence and presence of sodium fluoride, respectively (22). The 15-min reaction at 37°C, in a total volume of 150 µl, was started with the addition of 30 nmol of [3-14C]HMG-CoA (diluted with unlabeled HMG-CoA to a specific activity of 25 dpm/pmol; Amersham, Arlington Heights, IL). The reaction was stopped with the addition of 20 µl of 6 N HCl, and the labeled products were separated by thin-layer chromatography and determined by liquid scintillation counting (28).

Statistical analysis

Data were evaluated by standard statistical methods that included the one-way analysis of variance and comparisons of 95% and 99% confidence intervals for the means (29).
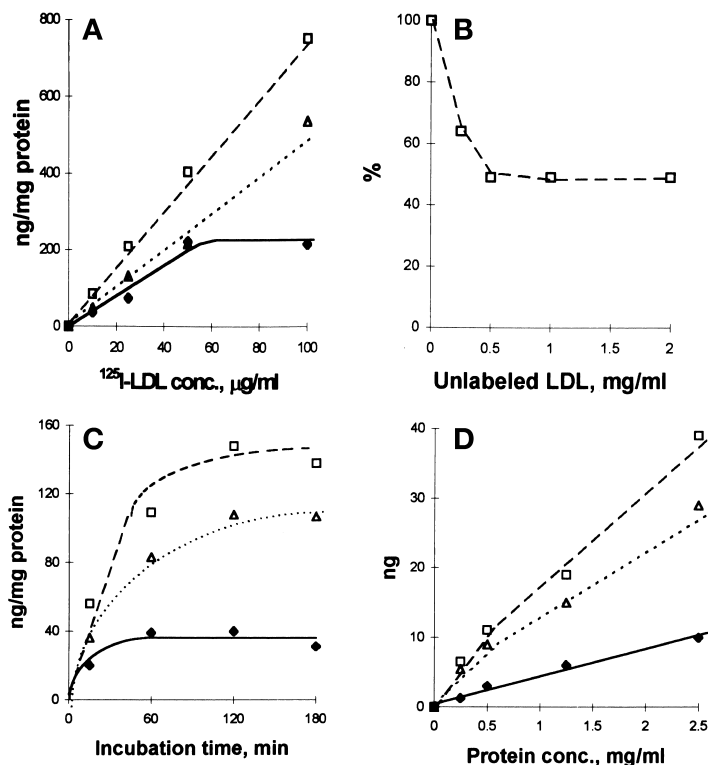
Binding of ^{125}I-LDL to Jejunal Mucosal Membranes

Fig. 1. Binding of LDL to membranes of jejunal mucosal cells. Receptor-mediated binding (solid diamonds) of ^{125}I-labeled LDL was determined as the difference between total binding (open squares) and nonspecific binding (open triangles), measured in the absence and presence of 40-fold excess unlabeled LDL, respectively. The effects of increasing ^{125}I-LDL concentrations (A), unlabeled LDL concentrations (B), incubation times (C), and membrane protein concentrations (D) were measured with 50 μg membrane protein, ^{125}I-LDL (25 μg/ml), unlabeled LDL (1 mg/ml) and 60-min incubation time, unless otherwise indicated.

RESULTS

Table 1 shows the effects of various experimental diets on cholesterol absorption, expressed as a percentage of dietary cholesterol (by the dual-isotope plasma ratio method) and as milligrams of cholesterol absorbed per day (calculated as dietary intake times percent cholesterol absorption). Supplementing the basal rodent (control) chow with 2% sitosterol, or 0.04% lovastatin, significantly ($P < 0.01$) decreased the amounts of absorbed cholesterol (-85%, and -63%, respectively). In contrast, the amount of cholesterol absorbed by cholesterol-fed animals was 27-fold higher than by control animals, even though they represented a smaller ($P < 0.01$) percentage of ingested cholesterol.

Cholesterol concentrations in jejunal mucosal cells are presented in **Table 2**. The cholesterol-supplemented diet increased the concentrations of esterified cholesterol in the jejunal mucosa nearly 4-fold ($P < 0.01$). However, the

moderate increase in total cholesterol concentrations was not statistically significant. No change in total and esterified cholesterol concentrations was observed in the jejunal mucosal cells of the sitosterol- and lovastatin-treated animals.

Receptor-mediated LDL binding represented about 50% of total LDL binding to mucosal cell membranes in the rat jejunum (Fig. 1). The regulation of LDL receptor function in the jejunal mucosal cells by various dietary manipulations is demonstrated in **Fig. 2**. The sitosterol and lovastatin diets, which decreased the amounts of cholesterol absorbed through the gut (Table 1), significantly upregulated receptor-mediated LDL binding to the mucosal membranes ($P < 0.01$). In contrast, the cholesterol diet,

TABLE 1.   Effects of various diets on cholesterol absorption in the rat

Diet	Absorption of Dietary Cholesterol	
	%	mg/day
Control	48.2 ± 3.1	3.25 ± 0.21
Cholesterol	17.2 ± 5.3[a]	87.16 ± 26.86[b]
Sitosterol	7.3 ± 1.1[a]	0.49 ± 0.07[a]
Lovastatin	17.8 ± 0.5[a]	1.20 ± 0.03[a]

Values represent means ± SEM. Four animals were measured per group.
[a] Significantly different from control, $P < 0.01$.
[b] Significantly different from control, $P < 0.05$.

TABLE 2.   Effects of various diets on cholesterol concentrations in rat jejunal mucosa

Diet	Cholesterol Concentration		
	Total[a]	Esterified	
	nmol/mg protein	nmol/mg protein	(% of total)
Control	70.3 ± 8.5	5.4 ± 0.7	(7.7)
Cholesterol	88.3 ± 8.1	21.4 ± 6.8[b]	(24.2)
Sitosterol	74.6 ± 10.6	4.6 ± 0.7	(6.2)
Lovastatin	91.1 ± 13.9	7.5 ± 1.6	(8.2)

Values represent means ± SEM. Six to 10 animals were measured per group.
[a] In addition to cholesterol, small amounts of sitosterol (5.5 ± 1.3 nmol/mg protein) were detected in sitosterol-fed rats. Total sterol (including sitosterol) concentrations in these animals were not significantly different from controls.
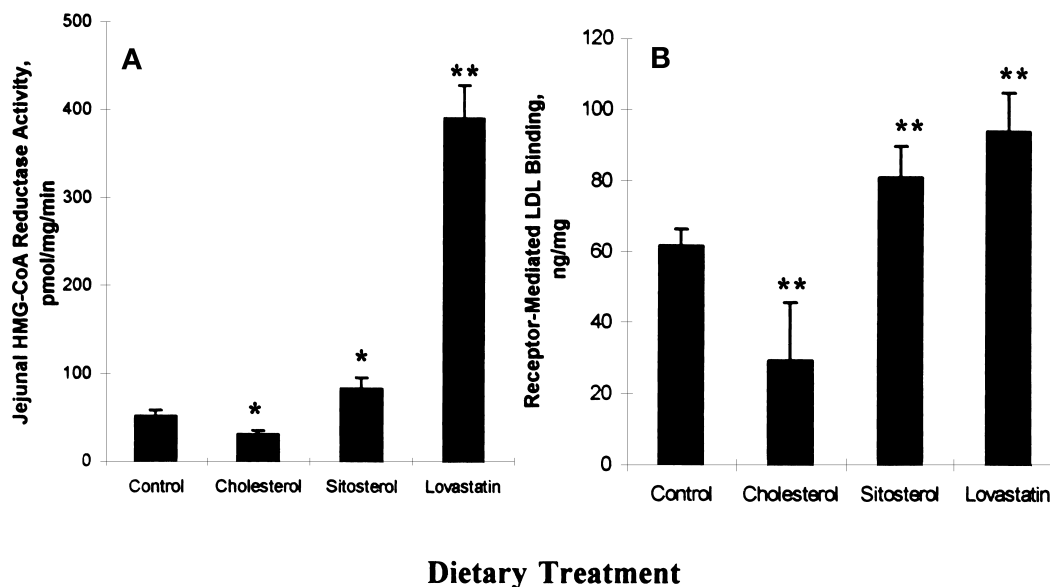[b] Significantly higher than controls, $P < 0.01$.

Fig. 2. Coordinate regulation of jejunal mucosal HMG-CoA reductase activity (A) with receptor-mediated LDL binding (B) by various diets. * $P < 0.05$ versus control diet. ** $P < 0.01$ versus control diet.

which provided an increased flux of absorbed cholesterol into the jejunal mucosal cells, significantly downregulated it (-62%, $P < 0.01$). Figure 2 also shows that LDL receptor function in the mucosal cells of the jejunum was coordinately regulated with jejunal HMG-CoA reductase activities: both were upregulated by sitosterol and lovastatin diets, and downregulated by the cholesterol diet.

The administration of lovastatin stimulated total jejunal HMG-CoA reductase activity nearly 4-fold ($P < 0.01$, **Table 3**), but most of the enzyme was not functional. Only 23% of the total enzyme was expressed in the dephosphorylated, catalyzing form, compared with 52% in controls ($P < 0.05$). In contrast, both total and expressed HMG-CoA reductases were downregulated by the cholesterol diet and upregulated by the sitosterol diet. In consequence, expressed enzyme in the cholesterol- and sitosterol-fed groups represented a constant percentage of total enzyme (not significantly different from that of the control group).

TABLE 3. Total and expressed microsomal HMG-CoA reductase activities in rat jejunal mucosa

Diet	HMG-CoA Reductase Activity	
	Total	Expressed
	pmol/mg/min	*%*
Control	51.5 ± 6.4	52 ± 8
Cholesterol	30.6 ± 4.5^a	43 ± 6
Sitosterol	82.4 ± 13.0^a	40 ± 8
Lovastatin	391.6 ± 38.3^b	23 ± 9^a

Values represent means $\pm$ SEM. Six to 10 animals were measured per group.

[a] Significantly different from control, $P < 0.05$.
[b] Significantly different from control, $P < 0.01$.

DISCUSSION

Results of this study showed for the first time that the parallel regulation of HMG-CoA reductase activity and LDL receptor function, which is well recognized in the liver, also exists in the jejunum. Both parameters respond to dietary and endogenously synthesized cholesterol, the two major sources of cellular cholesterol in the jejunal mucosa.

Sitosterol is a known inhibitor of cholesterol absorption (30–32), whereas lovastatin is better recognized as a competitive inhibitor of HMG-CoA reductase (33). Lovastatin was reported to inhibit cholesterol absorption in cholesterol-fed rabbits (34). In this study, lovastatin significantly inhibited cholesterol absorption in the absence of cholesterol feeding, via a mechanism that is still unclear. Lovastatin also inhibited the expression of jejunal HMG-CoA reductase activity as a dephosphorylated, active enzyme protein. This inhibitor, which shares some structural similarities with the natural substrate of HMG-CoA reductase, can bind to the enzyme protein and may interfere in the dephosphorylation (activation) step. However, the smaller rate of reductase activation is compensated for by the marked (4-fold) induction of total HMG-CoA reductase activity, which reflects both the functional and inactivated reductase proteins. The unusually large increase in total HMG-CoA reductase activity in lovastatin-fed animals may be the combined effect of compensation for both decreased absorption and competitive inhibition of HMG-CoA reductase. These compensatory mechanisms provide adequate amounts of endogenously formed cholesterol, and partly explain the resistance of the rat to the plasma cholesterol-lowering effect of lovastatin, which is frequently seen in humans treated with this drug.

Sitosterol inhibits cholesterol absorption by competing

with cholesterol for solubilization in the micelles prior to absorption (31, 32). Because sitosterol does not directly affect the activity of HMG-CoA reductase (22), the significant induction in HMG-CoA reductase activity in sitosterol-fed rats is likely due to an indirect effect of the potent effect of decreased cholesterol absorption. As seen in the cholesterol-fed animals, both total and expressed HMG-CoA reductase activities were stimulated so that the percentage of expressed enzyme was not significantly changed.

It should be noted that the large amounts of cholesterol absorbed through the gut of cholesterol-fed rats were calculated without including reabsorption of increased biliary cholesterol (35) and endogenous cholesterol from sloughed off mucosal cells. Cholesterol flux through the small intestine of cholesterol-fed animals was 27-fold higher than in chow-fed animals. Yet, HMG-CoA reductase and LDL receptor function were decreased by only 40–50%. This suggests that only part of the cholesterol fluxing through the intestine is entering regulatory metabolic pools of cholesterol in the absorptive cell. The increased amounts of esterified cholesterol further suggest that other enzymes, such as acyl-CoA:cholesterol acyltransferase, may play a role in preventing more significant changes in the tested parameters. However, the observed changes in HMG-CoA reductase and LDL receptor function are mechanisms that contribute to (if not accounting for all) cholesterol homeostasis in the mucosal cells of the jejunum.

The inverse relationship between amounts of cholesterol ingested and HMG-CoA reductase activity in the jejunal mucosa of cholesterol-fed rats could not be observed in earlier studies without concomitant feeding of bile acids (14). Downregulated total and expressed jejunal HMG-CoA reductase activities with cholesterol feeding alone was observed in this study without the effect of 5% corn oil, which was used in earlier studies with cholesterol feeding (14). Dietary fat has been shown to be inversely related to the mass of absorbed cholesterol (36), and thus may have prevented the decrease in jejunal HMG-CoA reductase activity in the earlier reports.

The question of whether cholesterol absorption is a regulated process is often raised without clear answer. The observation that the percentage of absorbed dietary cholesterol decreased when there was an increased flux of dietary cholesterol suggests that the decrease could be due to the limited capacity of micelles to solubilize all the ingested cholesterol. Studies (35, 37) have demonstrated a close relationship between cholesterol absorption and biliary secretion, and suggested that apolipoprotein E may play a critical role in regulating both biliary secretion and intestinal absorption of dietary cholesterol.

In brief, HMG-CoA reductase activity and mucosal LDL receptor-mediated uptake of cholesterol are coordinately regulated in response to diminished or increased fluxes of dietary cholesterol into the jejunal mucosa, so that mucosal cholesterol concentrations remain constant. When endogenous cholesterol synthesis is inhibited by a competitive inhibitor of HMG-CoA reductase (lovastatin), the compensatory stimulation of total HMG-CoA reductase protein and coordinate upregulation of LDL receptor function are mechanisms to provide adequate amounts of cellular cholesterol.

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